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Metabolism and Nutrition

Amino acid digestibility of larval meal (*Musca domestica*) for broiler chickens

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ABSTRACT

Work was undertaken to investigate the potential use of house-fly (*Musca domestica*) larvae reared on broiler manure as a source of nutrition for poultry production in the UK. Nutritional analysis showed that larvae have a high (>45% dry wt.) protein content and a favorable amino acid profile that is rich in key amino acids such as lysine and methionine. A broiler digestibility trial was carried out to determine the apparent ileal digestibility coefficients (AIDC) and true ileal digestibility coefficients (TIDC) of amino acids (AA) from insect larval meal (ILM) from *M. domestica* and fishmeal (FM) in broiler chickens. This was calculated using multiple linear regression technique based upon three inclusions of each protein source in a semisynthetic diet. One hundred and forty four day-old male (Ross 308) broilers were fed from hatch on a commercial starter diet for 20 days. Experimental diets were fed from day 21 to 28 and feed intakes were measured daily. On day 28 the trial was terminated, ileal digesta was collected for the determination of AIDC and TIDC of AA and inflammatory responses (gizzard erosion and eye discharge) were measured. No significant differences were observed in digestibilities between protein sources for any AA. Furthermore, ILM feeding did not induce gizzard erosion or eye discharge at any inclusion. These results provide strong evidence to suggest that ILM of the common house fly can provide a successful alternative protein source to FM in broiler diets.

Key words

Amino Acid; ileal digestibility; Broiler; House fly; Insect meal; Musca domestica;

Abbreviations

Amino acid, AA; Apparent ileal digestibility, AID; Apparent ileal digestibility coefficients, AIDC; Acid hydrolysis (Oil B), AH; Body Weight Gain, BWG: Crude Protein, CP; Feed Conversion Ratio, FCR; Feed Intake, FI: Fishmeal, FM; Insect larval meal, ILM,; True ileal digestibility, TID; True ileal digestibility coefficients, TIDC;

INTRODUCTION

A rising global population and growing appetite for animal products puts pressure on the supply of high quality proteins for animal production. Certain insects can be mass produced presenting an opportunity to alleviate reliance upon crop and animal products for livestock production. Whilst commercial scale production has already been achieved, relatively little is known of the nutritional value of insect meal for individual livestock species.

The rearing of houseflies for livestock feed has been researched since the early 20th century (McHargue, 1917) with comparisons of quality and nutritional value being discussed in the mid 1970's (DeFoliart, 1975) when poultry manure was evaluated as a substrate for rearing *Musca. Domestica* (common housefly) (Calvert et al., 1969, Calvert et al., 1970, Morgan et al., 1970, Miller et al., 1974, Teotia and Miller, 1974). More recent publications reporting the potential use of insects in poultry nutrition are based upon trials conducted in Asia, Africa, China, US and EU (Hwangbo et al., 2009, Veldkamp et al., 2012, Van Huis, 2013, Makker et al., 2014). However, the use of manure as a feeding substrate for housefly in industrialised countries has received less attention to date with exceptions of Pretorius (2011) who supported the production of insects on poultry manure for feeding to poultry as a circular economy. This is perhaps due to concerns related to their pest status and the safe use of insects reared on manures as compared to the black soldier fly (*Hermetia illucens*) that is able to grow on a wider range of vegetable and animal waste streams (Zheng et al., 2013).

Insects for use in animal nutrition have been gaining increased commercial interest since the recent EU regulation (2017/893) which has permitted insect meal to be fed in aquatic diets. It is expected that this will then be allowed in monogastric diets from as early as 2020 (ABN:AMRO, 2017). However few commercially relevant insect studies have been carried out to understand the nutritional characteristics and in vitro effects of feeding the novel

ingredients. For instance, insects are relatively high in chitin which can account for up to 8% (w/w) of the total CP content when calculated by N x 6.25. Chitin is a fibrous amino polysaccharide and therefore is hypothesised to provide similar gizzard stimulation as ingestion of coarse fibres from oat hulls and sugar beet pulp which have previously been shown to increase gastric acid secretion, gizzard activity and thereby lowering the pH of gizzard contents and in some cases causing gizzard erosion (Jiminéz-Moreno *et al*., 2009). Gizzard scoring was thus incorporated in this study to compare the effect of feeding high levels of insect meal to broilers. Eye discharge has also been recorded as a measure of the presence of allergenic conjunctivitis. Insects have been reported to contain similar allergenic compounds as shellfish which may stimulate an allergic reaction in both animals and humans consuming animals which have been reared on insects (EFSA Scientific committee, 2015).

The aim of this study was to understand amino acid (**AA**) digestibility of insect larval meal (**ILM**), as part of a wider feasibility study in which efforts were undertaken to understand the risks and value of this novel protein in livestock feeding. The ILM used was reared on poultry manure to understand the risks and values in this circular economy. Processing followed standards set out in European regulations and was found to be suitable to reduce microbial risks that were outlined in the risk assessment and were comparable to those outlined in the recent publication of our colleagues (Charlton et al., 2015).

The value in formulating livestock diets based on digestible AA content has long since been acknowledged (Rostagno et al., 1995 and Mosenthin et al., 2000), therefore this work provides vital information that underpins the development of appropriate diet formulations and estimations of commercial value.

MATERIALS AND METHODS

The study was carried out at the Brackenhurst Campus of Nottingham Trent University (UK). Institutional and UK national NC3R ARRIVE guidelines and European directive 2010/63/EU for the care, use and reporting of animals in research (Kilkenny *et al*., 2010) were followed and all experimental procedures involving animals were approved by the University's College of Arts and Science ethical review committee and the Food Standards Agency requirements for feeding of a non-approved feed material (ILM) to poultry.

Insect Larval Meal

The ILM was derived from *M. domestica* larvae reared on poultry manure and was produced by Grantbait Ltd., East Yorkshire, UK. It was subsequently processed using a method in alignment with the method 7 as set out in the EU processed animal proteins regulations (EC 142/2011, annex IV chapter III) in which microbial limits are outlined. Larvae were separated from the growth substrate before pupation and gut cleared on sand, the kill step consisted of submersion in boiled water before being dried (air-dried at ambient temperature for 12 hours, followed by 65°C for 3 hours). Whole larvae were then oven cooked for 40 minutes in a fan-assisted oven preheated to 95°C and ground to ensure biological risks were mitigated, Salmonella spp., *E. Coli* and Enterobacteria including coliforms were analysed on processed ILM for animal trials and were found to be below feed material limits as set out in animal feeding regulations EU directive 2002/32/EC as were other undesirable components. Sufficient quantity was produced for a broiler digestibility study in which the ILM was compared to a commercially available fishmeal (**FM**) (UFI Ltd, Grimsby (UK)) in order to understand the digestible AA levels using a multiple linear regression (as described in Batterham et al., 1979) with three feeding levels of each protein source, previously shown to be sufficient for analysis (Short et al., 1999; Rodehutscord et al., 2004).

Animals and housing

One hundred and forty four day-old male Ross 308 broilers were obtained (PD Hook Hatcheries Ltd, Cote, Oxford, UK) from a parent flock aged forty weeks. Ross 308 chicks were randomly allocated to wire mesh pens bedded on shavings and were housed in groups of six until day 21. On day 21, birds of a similar weight were re-housed in groups of four; unusual weight birds (+/- 100g of the mean weight) were removed from the trial. Each treatment was fed to six replicate pens of four birds. Pens were 0.64 m^2 with feed provided in 30 cm troughs and water via two nipple drinkers per pen. Prior to the trial period (day 1 to 21), chicks were fed a commercial starter, wheat: soyabean meal pelleted diet (Table 1), formulated to be sufficient in energy, AA, vitamins and minerals (228 g/kg of crude protein (**CP**); 12.8 MJ/kg metabolizable energy). At day 21 the birds were assigned to trial diets. Between days 21 and 28, feed intake was measured. At all times, feed and water were provided on an *ad libitum* basis and care was taken to ensure birds ate and drank on day 1. During the trial period the birds were kept under artificial light for twenty three hours per day, with one hour of dark on day 1 increasing by an hour of darkness each day until day 6. Six hours of darkness (22:00-24:00 and 02:00-06:00) was then maintained for the remainder of the study. The room was thermostatically controlled to produce an initial temperature of 32°C on day 1 and reduced in steps of 0.5°C per day, reaching 21°C by day 14. Temperatures were recorded daily from different areas of the unit and health checks made twice daily. Prior to culling on day 28, the birds were fed fresh diet for a minimum of 30 minutes to ensure gut fill. Post weighing, birds were assessed for potential allergic response by the presence of eye discharge. Birds were then culled by cervical dislocation. The weight of each carcass was recorded and the gizzard removed from one bird per pen, emptied and washed before scoring for erosion. The ileal region of the gut was dissected out from the Meckel's diverticulum to the ileal-caecal junction. Ileal digesta was collected to determine the apparent ileal digestibility (**AID**) and thus the true ileal digestibility (**TID**) using the multiple linear

143 approach as set out by Short et al., 1999). Digesta was pooled per cage (four birds) and sent
144 for AA analysis. Apparent ileal digestibility coefficients (**AIDC**) and true ileal digestibility
145 coefficients (**TIDC**) are communicated in this paper for brevity.

146

147 ***Treatment diets***

148 The six treatment diets were designed to allow determination of AA digestibility of ILM and
149 FM by regression analysis (Batterham et al., 1979; Short et al., 1999) and to enable a
150 comparison between these two protein sources. All diets were semisynthetic, in mash form
151 including 20, 40 or 60% ILM (w/w) or FM as the sole protein source, with the remaining diet
152 made up of a 50:50 mix of corn starch and glucose. All treatments contained a vitamin and
153 mineral premix (50 g/kg) designed for semisynthetic diets (Target Feeds, Shropshire, UK),
154 soyabean oil (50 g/kg) to bind the diet and reduce dustiness and titanium dioxide (5 g/kg) as
155 an indigestible marker. All experimental diets were manufactured on site at Nottingham
156 Trent University. Protein ingredients were ground on a Retsch mill (Retsch-Allee, Haan,
157 Germany) fitted with a 3mm screen and diets were then mixed using a commercial ribbon
158 mixer (Rigal-Bennett, UK) for 8 minutes to ensure homogeneity. All diets were stored at
159 ambient temperature.

160

161 ***Inflammatory assessment***

162 Eye discharge assessment was carried out at day 28 by a single competent individual who
163 assessed presence or absence of discharge. Gizzards were removed from one bird per pen,
164 emptied and washed with distilled water before scoring for erosion of the lining on a 5 point
165 scale, amended slightly from that used by Okazaki et al. (1983) to increase the scoring range,
166 as detailed below:

1 No erosion

2 Light erosion (roughness of koilin layer)

3 Modest erosion (roughness and gaps)

4 Severe erosion (roughness, gaps and ulcers on stomach wall showing slight haemorrhaging)

5 Extreme erosion (roughness, gaps and haemorrhagic ulcers on stomach wall and separation of epithelia from stomach wall)

Chemical analyses and calculations

For samples of diets, dry matter (**DM**) was determined in triplicate by weighing approximately 500 mg samples that were dried to a constant weight at 100 °C in a forced air convection oven. Due to their small sample size and collection directly into plastic containers, digesta samples were frozen and then freeze-dried to a constant weight when determining dry matter. The concentration of titanium dioxide (employed as an inert marker) in diet and digesta samples was determined using the spectrophotometric method described by Short et al. (1996). Crude protein was calculated as N x 6.25. AA analysis was conducted as follows: briefly, diet and digesta samples (~500 mg) were freeze-dried before being milled, and hydrolysed in duplicate using both 6N HCl and 4M NaOH at 110 ºC under vacuum for 22 hours. After hydrolysis the samples were allowed to cool before extraction with 1 ml of de-ionized water. Extracts were filtered through 0.22 µm PTFE filters before a 10-fold dilution with water and analysis by liquid chromatography – UV detection (LC-UV). A known protein (lysozyme) and a known reference sample (fishmeal) were concurrently hydrolyzed and analyzed with each batch as quality controls. Detection by LC-UV used the "Aracus" fully automatic AA analyser (MembraPure GmbH, Berlin, Germany) with an ion

exchange chromatography column (125 mm x 3 mm) to separate each AA before post column derivatization with ninhydrin. Detection of acids by UV was monitored at 570 nm and 440 nm. Total chromatographic run time was 2.5 hours per sample. Each AA was quantified using a certified standard mix of AA (Sigma-Aldrich, Gillingham, UK) injected alongside the analysis. Tryptophan concentration was calculated from the base hydrolysis; all other concentrations were calculated from the acid hydrolysis.

Using the titanium dioxide measurements, the AA results were used to calculate AID using the following equation:

$1-(aa_{dig} * marker_{feed}) / (aa_{feed} * marker_{dig})$

Where:

aa_{dig} represents the AA content of the digesta

$marker_{feed}$ represents the titanium concentration in the diet

aa_{feed} represents the AA concentration in the diet

$marker_{dig}$ represents the titanium dioxide concentration in the digesta

The AA content of protein sources and digesta was evaluated following methods set out in EC 98/64/EC. The AID content of the diets was regressed against the rate of inclusion of the ILM and FM. The linear regression was then extrapolated to a rate of inclusion of 100% (or 1000 g/kg) protein (Rodehutscord et al., 2004). This gave a figure for AID of the protein sources for each AA measured. Dividing this figure by the total content of the specific AA in the protein gave an AIDC. TID was then calculated by addition of the intercept of the extrapolation to the AID values for each AA to account for endogenous losses as previously described by Short et al. (1996; 1999). The figure for TID was then divided by the total content to provide TIDC values.

Statistical Analysis

All data were exported to SPSS v.22 (IBM statistics, 2012) and after KS testing to confirm normality. The mean values for the AIDC and TIDC for each protein source were separated by paired t-test and were considered significant at $P<0.05$.

RESULTS

Diet formulation

The starter diet was fed prior to the study period, ingredients and calculated analysis is shown in Table 1. Experimental diets were formulated following triplicate analysis of the ILM and FM for DM, CP, crude fibre (**CF**), acid hydrolysis (**AH**), ash and total AA composition. This analysis is shown in Table 2 and experimental diet formulation and analysis shown in Table 3. ILM analysed higher in DM, CF, AH but lower in CP and ash as compared to FM. AA compositions were similar between the two protein sources with ILM higher in key AA such as Cys, Try and Tyr but lower in Lys, Met and Val on an as fed basis.

Bird Performance

Bird performance was comparable to other digestibility trials at this facility. There were no significant differences for the two protein sources in any performance parameters measured over the study period (Table 4); initial body weight (**BW**) (day 21), final BW (day 28), body weight gain (**BWG**) or feed intake (**FI**). No eye discharge of any kind was recorded for any bird at any point during the trial. Gizzard erosion was higher in birds fed ILM ($P<0.05$; Table 4) compared to FM but no severe or extreme erosion was seen in any inclusion for either protein source.

Amino acid digestibility

The determined values for AIDC of the AA are shown in Table 5. There were no significant differences seen between protein sources (P=0.119) (Table 5). FM values were similar to those previously recorded in the facility for this age of bird. Lys, Met, Try and Cys are all numerically higher for ILM with respective values of 0.87, 0.88, 0.81 and 0.82 versus respective values of 0.86, 0.86, 0.55 and 0.79 for FM. Other AIDC values for the different protein sources were either identical, or very similar.

The TIDC values for each protein source are shown in Table 5. The TIDC values for ILM and FM did not significantly differ for any AA (P=0.385).

DISCUSSION

Proximate analysis of the protein sources showed the full fat ILM had a higher AH and lower CP than FM, as the oil has not been removed from the ILM through further processing. The removal of fats would result in a higher CP content and lower AH potentially providing an even better replacement for high protein FM than full fat ILM. Defatting is suggested for the meal obtained from housefly and other species as a way to improve their quality Henry et al. (2015). This would be especially relevant for diet formulation as the oil content would limit the inclusion of ILM for diet production constraints.

Nutritional composition of the protein sources was comparable to publically available sources such as feedipedia, supported by INRA (Heuzé and Tran, 2015; Heuzé et al., 2015) and recent FAO publications (Makkar et al., 2014). This would suggest that the method for production and processing of ILM used in this study is suitable to produce a representable

sample for evaluation. The nutritional information on house fly larvae in the feedipedia data sheet is compiled from more than 80 sources by Heuzé and Tran (2015). Differences in reported nutritional profiles of *M. domestica* are potentially due to rearing conditions and substrate used; this has not been evaluated for this study. However the authors have other experiments due to be published which discuss the effect of rearing environment and diet on nutritional profile of *M. domestica* (Fitches et al, personal communication).

The values for AIDC and TIDC were similar to those expected for FM in this trial facility for the same age of birds. Values were also close to those published in literature reviews (Lemme et al., 2004; Kim et al., 2012) although Cys and Try AID values were different in Ravindran et al., (2005) with 0.57 and 0.77 for Cys and Try respectively versus 0.79 and 0.55 in this study. This may be due to differences in AA analysis, digestibility methodology and difficulties in accurately analysing these AA. It is well known that digestibility values obtained for a raw material will depend on the specific method used (Kong and Adeola, 2013; Masey O'Neill et al., 2014). Digestibility coefficients for larval meal were slightly lower than those reported by Hwangbo et al., (2009) for broiler chickens fed on house fly meal. This may be due to the processing that was used, a slow drying process of 55°c over 24 hours (Hwangbo et al., 2009). Our process followed the EU requirements for processed animal proteins and so a minimum temperature was maintained for 20 minutes to ensure microbial parameters were met. This process would have likely resulted in more maillard reactions and therefore reduced protein digestibility. Reported apparent digestibility coefficients for essential AA were 0.976, 0.956, 0.956 and 0.945 for Lys, Met, Arg and Val respectively compared to AIDC values of 0.87, 0.88, 0.88 and 0.81 documented from our study. These differences may also be partly due to methodology used and the age of bird at time of collection. Hwangbo et al. also used adult birds at 28- 35 days of age compared to our trial which terminated at 28 days of age.

Gizzard erosion was higher in ILM treatments than FM and this may be due to the presence of chitin in the ILM especially at the higher inclusion levels. Alternatively this may be as a result of the presence of biogenic amines, as the heating of histidine and lysine can produce gizzorosine which stimulates the secretion of acid and can increase gizzard lesions (Gjevre et al., 2013). However, in commercial practice it is unlikely that inclusion of ILM would go above 10%. Even the highest inclusion of ILM in this study (60%) did not produce a gizzard score above light erosion only (score less than 2), so a 10% inclusion level is very unlikely to lead to a detrimental effect in practice. However, this should be monitored in further studies. Hossain and Blair (2007) found no negative impacts upon the performance of broilers fed on diets containing up to 7.5% (w/w) of crustacean derived chitin, reporting true chitin digestibility to be 0.87.

Although some insect meals have been shown to include tropomyosin which has allergenic properties similar to shellfish (Charlton et al. 2015), there were no observed allergenic reactions observed in this study, which suggests that either this molecule is not present in this meal, or at levels which are not deleterious to the bird.

M. domestica larvae have been proven to be suitable ingredients in the diets of poultry (Zuidhof et al., 2003) when used to replace up to 50% of FM or soyabean meal (Akpodiete and Inoni, 2000; Hwangbo et al., 2009; Okah and Onwujiariri, 2012). Rearing insects on poultry manure for animal feeding has been previously reviewed as a means to convert nitrogenous waste into high value protein for livestock (Calvert, et al., 1970; El Boushy et al., 1985; El Boushy, 1991; Hwangbo et al., 2009; Pretorius, 2011). However, as a feed material the substrate used in this study may be of higher risk as compared to conventional protein sources. The EFSA committee report published in 2015 ‘insects as food and feed’ highlighted the need for further research where manures and wastes are utilised as substrates for insect production. Consumer perception was also discussed in the EFSA review as a potential

barrier in western countries and many studies including those supported by the FAO and Wageningen University (Van Huis et al., 2013) are working towards improved global protein sustainability and consumer awareness. In a recent study, two thirds of both stakeholders and members of the general public questioned were generally favourable towards the use of insects to feed production animals (Verbeke et al., 2012).

As the world population nears 9 billion it will become increasingly more costly to produce animal protein such as poultry, pork and fish as feed protein resources become more in-demand and production of vegetable proteins and fishmeal cannot fulfil the requirement. The use of insects in these diets can therefore be of benefit and housefly meal has been shown to have the potential to reduce the cost of poultry production by as much as 75% in Africa (Akpodiete and Inoni, 2000) and to significantly improve performance ($P<0.05$) when it replaced fishmeal by up to 50% (Okah and Onwujiariri, 2012).

Previously, *M. domestica* has been given a nutritive value between that of FM and soyabean meal when fed to broiler chicks (Ocio and Rey, 1979). Teotia and Miller (1974) suggested that for growing chicks, house fly pupae are a good source of limiting AA, particularly Arg, Lys, and Met when compared to soyabean meal. In our study we have shown that processed insect meal has comparable amino acid digestibility coefficients to that of commercial fishmeal providing further evidence that insects offer significant potential for exploitation by the animal feed industry

Processed insect meal is now allowed to be used in feeds for aquaculture (EU regulation 2017/893) and has been shown to provide an alternative to the use of fishmeal (Henry et al., 2015), with *Dipteran* (fly species) reportedly having an AA content closest to FM (Barroso et al., 2014). With this change to legislation it is expected that insect meal will be permitted into

the diets of non-ruminants in the near future. Providing the industry continues to carry out research to help understand this novel material.

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473 Table 1. Starter feed diet formulation, g/kg except where stated

Ingredients		Calculated composition (of diet, all expressed as total)	
Wheat	541.0	ME, MJ/kg	12.8
Soyabean meal	260.0	Crude Protein	228.0
Fishmeal	50.0	Crude Ash	50.0
Extruded horse beans	40.0	Crude Fibre	30.0
Extruded rapeseed	35.0	Crude Oil & Fats	55.0
Soyabean oil	30.0	Calcium	8.0
Maize gluten	15.0	Lysine	14.5
Dicalcium phosphate	12.0	Methionine	4.7
Limestone	10.2	Methionine eq. value	7.0
Sodium bicarbonate	1.9	Phosphorus	6.0
Sodium chloride	1.8	Sodium	1.5
Vitamin and mineral premix[1]	3.0		
Maxiban[2]	0.1		

474 Vitamin and mineral premix[1]: Vitamin: A, 10,000 IU; Vitamin D3, 2,000 IU; vitamin D 25-HY-D 2000 IU
475 vitamin E 75 IU, Zinc sulphate, monohydrate (E6−Zinc) 277.78 mg. Manganous oxide (E5−Manganese) 161.29
476 mg. Ferrous sulphate, monohydrate (E1−Iron) 133.34 mg. Cupric sulphate, pentahydrate (E4−Copper) 60.00
477 mg. Calcium iodate, anhydrous (E2−Iodine) 3.23 mg. Sodium selenite (E8−Selenium) 0.67 mg.

478 [2]Supplied 50.00 mg of Narasin and 50.00 mg of Nicarbazin per kg of diet.

479

480 Table 2. The analysed proximate and total amino acid content of the experimental protein sources

	Protein sources[1]	
Proximate analysis (g/kg as fed)	Fishmeal	Insect meal
Dry Matter	908	920
Crude Protein	645	533
Crude Fibre	4.5	59
Acid Hydrolysis (Oil B)	97	203
Ash	162	65
Amino acids (g/kg as fed)		
Alanine	46.61	34.73
Arginine	42.11	30.16
Aspartic	65.88	62.08
Cysteine	14.83	17.38
Glutamic acid	92.99	84.41
Glycine	53.54	28.43
Histidine	16.91	18.17
Isoleucine	31.51	22.62
Leucine	54.44	38.30
Lysine	56.94	44.92
Methionine	22.59	15.77
Phenylalanine	27.60	37.80
Proline	31.79	23.82
Serine	16.78	15.82
Threonine	39.49	33.20
Tryptophan	23.90	41.00
Tyrosine	24.23	40.74
Valine	33.31	26.97

481 [1] Fishmeal, commercial Fishmeal; Insect meal, ground full fat *Musca domestica*

Table 3. Experiment diet formulations (g/kg diet)

	Dietary treatments					
	20% Fishmeal	40% Fishmeal	60% Fishmeal	20% Insect meal	40% Insect meal	60% Insect meal
Fishmeal	200	400	600			
Insect Meal				200	400	600
Corn Starch	347.5	247.5	147.5	347.5	247.5	147.5
Glucose	347.5	247.5	147.5	347.5	247.5	147.5
Soyabean Oil	50	50	50	50	50	50
Vitamin and Mineral Premix[1]	50	50	50	50	50	50
$Ti0_2$	5	5	5	5	5	5
Analysed diet composition						
Dry Matter	927.18	937.05	939.46	931.48	945.98	960.79
Crude Protein*	141.56	282.14	433.10	118.76	223.31	355.18
Fat	67.17	67.98	66.32	67.7	66.49	66.83
Gross Energy (MJ/kg)**	17.94	18.30	18.92	18.95	20.29	22.05
Ash	70.37	103.58	143.69	45.08	60.70	79.29

[1]Vitamin and mineral pre-mix provided the following (per kg of diet): phosphorus, 5 g; magnesium, 90 mg; calcium, 7.5 g; sodium, 1.5 g; copper, 0.6 mg (as copper sulphate); selenium, 160 µg (as selenium BCP); vitamin A, 7500 IU; vitamin D3, 1500 IU; vitamin E, 10 IU (as α-tocopherol acetate); vitamin B1, 5 mg; vitamin B2, 4 mg; vitamin B6, 4 mg; vitamin B12, 10 µg; pantothenic acid, 9 mg; folic acid, 1.5 mg; biotin, 150 µg; choline, 1500 mg.

*Analysed by DMS ** Analysed by PAS

489 Table 4. Performance results of broilers fed experimental protein sources measured from 21 to 28 days

	Protein sources[1]		
	Fishmeal	Insect meal	P-Value
D 21 BW (g)	1109	1083	0.801
D28 BW (g)	1475	1453	0.528
BWG D21-28 (g/d)	366	371	0.844
FI/bird (g/bird)	681	650	0.228
Gizzard score[2]	1.06	1.56	0.006

490 [1] Fishmeal, commercial Fishmeal; Insect meal, ground full fat *Musca domestica*

491 [2] Gizzard scoring on a 5 point scale adapted from Okazaki et al., 1983

492 D, days; BW, Body weight; BWG, body weight gain; FI, feed intake

493 Table 5. The coefficient of apparent ileal digestibility (AIDC) and true ileal digestibility (TIDC) of amino acids

494 in the experimental protein sources determined in 28 day old broilers

	AIDC Protein sources [1]		TIDC Protein sources[1]	
Amino acids (g/kg)	Fishmeal	Insect meal	Fishmeal	Insect meal
Alanine	0.83	0.85	0.92	0.89
Arginine	0.90	0.88	0.98	0.92
Aspartic	0.74	0.86	0.87	0.90
Cysteine	0.79	0.82	0.97	0.95
Glutamic acid	0.82	0.86	0.90	0.90
Glycine	0.76	0.77	0.83	0.83
Histidine	0.82	0.85	0.91	0.89
Isoleucine	0.81	0.80	0.91	0.85
Leucine	0.84	0.83	0.94	0.88
Lysine	0.86	0.87	0.93	0.90
Methionine	0.86	0.88	0.93	0.91
Phenylalanine	0.78	0.88	0.88	0.92
Proline	0.73	0.69	0.83	0.79
Serine	0.79	0.82	0.93	0.91
Threonine	0.78	0.78	0.90	0.87
Tryptophan	0.55	0.81	0.74	0.91
Tyrosine	0.88	0.91	0.99	0.95
Valine	0.81	0.81	0.91	0.87

495 [1] Fishmeal, commercial Fishmeal; Insect meal, ground full fat *Musca domestica*

496 No significant difference between protein sources for AIDC (P=0.119) or TIDC (P=0.385)

497